Applicants regard as the invention. The Applicant respectfully disagrees with the rejection as set forth in the Office Action.

Claims 1 and 11-21 recite that the oxygen scavenging packet comprises at least some amount of oxygen absorber and some amount of liquid oxygen uptake accelerator. The Applicant believes that it should not be forced to limit its claims by stating a particular amount of each of the above-listed elements. The Applicant welcomes any suggestions that the Examiner might have to forward these claims to allowance.

Section § 103 Rejections

Claims 1, 11, 13-15, 17-19, and 21 were rejected under 35 U.S.C. § 103 (a) as being unpatentable over U.S. Patent No. 4, 588,561 to Aswell (hereinafter referred to "Aswell"). Aswell does not teach the claimed ratios of absorber to accelerated and therefore and does not teach the unexpected results and benefits and speed of reduction of oxygen levels as seen by the use of these ratios. Aswell teaches that "water should be added to the composition at a level of about 60 to about 80 percent of the weight of the oxygen reactive composition." (Aswell, col. 4, lines 53-56). Aswell also teaches that the oxygen reactive composition contains from about 4.5 to about 6.0 grams (col. 4, lines 32-34) and that the amount of iron in the oxygen reactive composition is from about 25 to about 35 percent. (col. 4, lines 15-16). The calculation using these numbers shows that the amount of water to be used per gram of iron is on the order of 10 times that of the claimed invention.

By contrast, the invention as claimed differs from the prior art in its ability to reduce the oxygen concentration in a modified atmosphere package at a very fast rate. This increased rate is brought about by the addition of specific amounts of an oxygen accelerator comprising water to the iron-based oxygen absorber immediately before use of the oxygen scavenging

packet. Figure 11 of the present application shows that by practicing the claimed invention, two commercially-available packets injected with the claimed amount of accelerator of capable of reducing the oxygen level in a quart-sized jar from about 2 volume percent oxygen to nearly 0 volume percent oxygen in about 90 minutes.

Figure 11 also shows that two identical commercially available packets for which the accelerator was passably introduced partly reduced the oxygen level at all in the same time. The need for this fast oxygen removal rate is illustrated by D. A. Ledward in the attached article entitled, "Metmyoglobin Formation in Beef Stored in Carbon Dioxide Enriched and Oxygen Depleted Atmospheres,"[1] ("Ledward"). Ledward shows that meat undergoes irreversible discoloration and browns, *i.e.,* forms metmyoglobin, when myoglobin within the meat is subjected to certain oxygen percentages. Ledward's Figure 3 illustrates that metmyoglobin forms quickly on the muscle surface of meat in the presence of oxygen and that the amount of metmyoglobin peaks when the partial pressure of oxygen reaches 6 mm Hg at 0° Celsius and 7.5 mm Hg at 7° Celsius.[1] The Ledward article shows the importance of designing a system for the uptake of oxygen that can absorb oxygen rapidly enough to prevent metmyoglobin formation.

Claims 1, 12, 16, 17 and 20 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 4,820,442 to Motoyama et al ("Motoyama"). The Examiner stated that Motoyama differed from the claims of the present invention in that the claimed amount of oxygen scavenger are disclosed and that these amounts would be expected by one of ordinary skill in the art. However, because Motoyama does not teach the need for a quick reduction in the oxygen level as illustrated by the attached Ledward article, one skilled

[1] Ledward, D. A., *Journal of Food Science,* 35:33-37 (1970).

in the art would not be motivated by Motoyama to optimize the amounts of oxygen scavenger having no reason to do any optimization and thus would not arrive at the claimed invention. Specifically, in Table 1, Motoyama discloses oxygen content reduction after a period of **two days**. (emphasis added). Independent Claims 1 recites that the oxygen content reduction for the oxygen scavenging packets of the present invention are reduced to less than 0.5 vol. % after a period of no more than **90 minutes.** (emphasis added). The method claim 17 recites that the oxygen absorber is capable of reducing the oxygen content to about the same level in about the same time. The cited references of Motoyama and Nakoneczny do not teach or suggest such a rapid reduction of oxygen content. Because claims 11-16 and claims 18-21 depend directly on respective independent claims 1 and 17, dependent claims 11-16 and 18-21 should also be in condition for allowance.

Further, contrary to the assertion in the Office Action, the use of an oxygen uptake accelerator within a bibulous wick is not taught or suggested by Nakoneczny. Thus, it would not have been obvious to one of ordinary skill in the art to use an uptake accelerator contained in a bibulous wick as recited in the present application.

As discussed above and for the aforementioned reasons, the claims of the present invention are distinguished from the prior art. Therefore, in view of the discussion above, Applicants respectfully submit that all the claims presented in this application are allowable over the cited references of record. Examiner is invited to call the undersigned to discuss any other issues that may need to be resolved.

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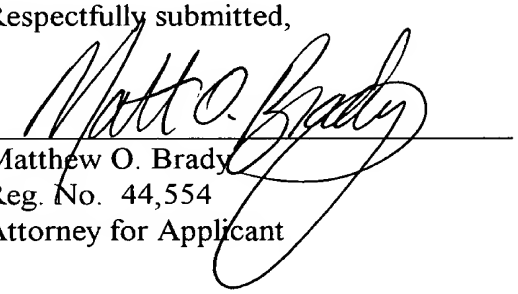
[1] *Ibid.*, p. 35.

The Commissioner is hereby authorized to charge Deposit Account No. 10-0447 (Order No. 47097-00716USC2) for any additional fees inadvertently omitted (except for the issue fee) that may be necessary now or during the pendency of this application.

Respectfully submitted,



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#3 IDS

Metmyoglobin Formation in Beef Stored in Carbon Dioxide Enriched and Oxygen Depleted Atmospheres

SUMMARY—A spectroreflectometric technique was used to determine the relative percentages of three myoglobin pigments, reduced myoglobin, oxymyoglobin and metmyoglobin at the surface of fresh beef. It was shown that, at constant humidity, the formation of metmyoglobin in beef was maximal at 6 ± 3 mm Hg of oxygen at 0°C and 7.5 ± 3 mm Hg at 7°C for semitendinosus muscles. Carbon dioxide concentrations of 10% and higher had negligible effect on the formation of metmyoglobin, provided the oxygen pressure was above about 5%. At high partial pressures of carbon dioxide, absorption of carbon dioxide increased and the pH of the surface decreased. In air, the formation of metmyoglobin varied widely from muscle to muscle.

INTRODUCTION

FOR TENDERIZING fresh beef by aging, it is necessary to hold the meat at temperatures above the freezing point for several days. Maximal tenderization is obtained at 0 to 2°C in about 14 days, shorter times being adequate at higher temperatures (Kuprianoff, 1964). At these temperatures bacterial spoilage is dependent on the initial population density. Extending storage life by using low relative humidities (Scott, 1936) leads to marked weight loss and discoloration. Haines (1933) and Scott (1938) extended the life of fresh meat by storage in selected atmospheres, low in oxygen and/or enriched with carbon dioxide. The present work was undertaken to determine if any undesirable color changes occurred in beef muscle stored at low oxygen and/or high carbon dioxide partial pressures, at a high relative humidity.

Any undesirable color changes will attain increased significance as the size of the joint decreases, thus if half or quarter carcasses are aged any surface discoloration will be relatively unimportant after butchering for retail consumption. Limitations on storage space often make this method of aging uneconomical and there is an increasing tendency to age in boneless, retail size cuts. Under these conditions surface discolorations are of major importance.

In sterile meat, of normal water content, the color is due mainly to the heme proteins myoglobin and hemoglobin. At a high relative humidity (99.3%), any discoloration of sterile, post-rigor meat is due to the oxidation of these pigments to the brown metmyoglobin and methemoglobin. (Myoglobin is the major colored protein in fresh beef and the color changes of myoglobin and hemoglobin are, to a first approximation, the same. Consequently the pigment states are generally analyzed in terms of myoglobin derivatives.)

Little objective work has been performed on the effect of carbon dioxide on color of fresh meat. Brooks (1933) found that up to 20% carbon dioxide (in air) had negligible effect on the oxidation of heme pigments in meat although 30% led to a slightly increased rate of oxidation, which he attributed to the reduced oxygen partial pressure. Brooks (1931, 1935) working with ox-blood (hemoglobin), and George et al. (1952a, 1952b) using pure horse heart myoglobin, found the rate of oxidation to be very dependent on the partial pressure of oxygen in the system. The rate was maximal at low partial pressure.

In meat the situation is more complex because (a) the oxidation is quasi-reversible as the enzymatic reduction of metmyoglobin to one of the reduced forms can occur (Stewart et al., 1965b), and (b) it is extremely difficult to determine, accurately and non-destructively, the pigment states at the meat surface. In the analysis of the pigment states in meat, extraction procedures are cumbersome; they destroy the sample analyzed and possibly cause changes in the relative proportions of the three pigments. For these reasons a spectroreflectometric method of analysis was preferred.

The three myoglobin pigments, both in meat and in solution, have an isosbestic point at 525 nm (Stewart et al., 1965b). Stewart et al. (1965a) estimated the percentage metmyoglobin at the surface of minced meat samples from the ratio of K/S values at 572 nm (an isosbestic point for reduced myoglobin and oxymyoglobin) to that at 525 nm. K/S is defined as

$$\frac{(1 - R\infty)^2}{2R\infty} \qquad \text{(Kubelka et al., 1931)}$$

where $R\infty$ is the reflectivity of an opaque sample of such a thickness that there is no further change in reflectivity when the thickness is increased further.

This technique corrects for any difference in the total concentration of myoglobin pigments in the sample, but does not adequately allow for differences in the scattering (S) and absorption (K) coefficients of the meat matrix itself. Either one or both these coefficients will vary with pH, fat content, surface geometry and water content of the sample, as well as with the incident wavelength. The effects are such that the ratios of K/S at 572 nm to K/S at 525 nm are unlikely to be constant for "pigment free meat."

Snyder (1965) attempted to overcome the problem by adjusting all his spectral curves to a common reflectance (in absorbance units) of $R_A = 1.0$ at 525 nm, and estimating the metmyoglobin content by the reflectance at 572 nm. Unfortunately his plot of R_A at 572 nm against percent metmyoglobin, for known mixtures of oxymyoglobin and metmyoglobin in an aqueous suspension of dried milk was not linear.

Using these data Snyder et al. (1967) found that when K/S at 572 nm was plotted against metmyoglobin concentration the predicted linear relationship was obtained. They also found, for 18 beef rounds of known pigment state, that determinations of surface metmyoglobin concentration from the ratio of K/S values at 572 and 525 nm and from the K/S value at 572 nm after adjustment of R_A at 525 nm to 1.0 were equally accurate although, conceptually, they considered the ratio method to be preferable.

EXPERIMENTAL

Preparation of sterile samples

Discs of 14 mm radius and 15 ± 1 mm thickness were cut under aseptic conditions from a semitendinosus muscle which had been removed from the carcass directly after slaughter. Muscle was extensively flamed and aged for 2 days at 0°C in a closed, sterile container. These discs were used in the storage experiments. In initial experiments heme pigments in some of these discs were converted to 100 or 0% metmyoglobin by the action of ferricyanide (1%) and dithionite (20%) respectively. Other discs were minced and adjusted to various pH values

in the range 5.3 to 6.8 with M HCl and M NaOH before the pigments were converted to 100 or 0% metmyoglobin.

Gaseous atmospheres

A continuous flow of air or nitrogen (both containing 10% carbon dioxide), maintained at a relative humidity of 99.3% by bubbling through towers containing 0.2*M* sodium chloride solution, was passed through the container holding the samples. The whole system was sterilized before use. Microbial contaminants in incoming gases were removed by passing the gases through cotton wool plugs. Each system was stored in a room kept at the appropriate temperature (0 or 7°C). Air controls were stored under similar conditions. At least 9 samples were stored in each container.

In some experiments meat slices of radius 38 mm were stored in closed, sterile, plastic containers leaving a headspace of 18 ± 1 cc. Headspace samples were removed through sub-a-seals. A flushing arrangement through the seal allowed the gas composition in the headspace to be adjusted at will.

Spectral analysis, gas analysis and pH

The reflectance spectra of the meat samples were recorded against a magnesium oxide standard on a Hitachi Perkin-Elmer Spectrophotometer, Model 139, with reflectance attachment. The range scanned was 380 to 770 nm. After storage all samples were exposed to air (R.H. 99.3%) for 2–3 hr, at the storage temperature, so that any reduced myoglobin at the surface was oxygenated while avoiding any detectable change in the surface concentration of metmyoglobin. Spectra of the exposed meat surfaces were then recorded at 8 ± 1° C. Samples were contained in stainless steel cups of appropriate dimensions.

Gas analyses were performed at regular intervals using a 25V Fisher Gas Partitioner calibrated with purified gases. One tenth ml samples of gas, extracted with SGE gas tight syringes, were used. Average equilibrium concentrations are reported.

Surface pH was measured with a surface electrode and Radiometer Model TTT IC Titrator and pH meter.

Heme pigment concentration was measured according to the method of Hornsey (1956).

RESULTS & DISCUSSION

Determination of metmyoglobin at the surface

In preliminary experiments, minced semitendinosus samples, of known pigment state and different pH values were analyzed. In these the ratio of K/S at 572 nm to K/S at 525 nm, for 100% and 0% metmyoglobin varied and there was slightly better consistency in the K/S values at 572 nm when R_A at 525 nm was adjusted to 1.0 absorbancy units. Results obtained with several intact semitendinosus samples of known pigment state were consistent with those obtained on minced samples.

For 18 samples the K/S values at 572 nm, after adjustment of R_A to 1.0 at 525 nm, were respectively 2.41 ± 0.19 (range 2.15–2.62) and 6.05 ± 0.18 (range 5.80–6.30) for 100% and 0% metmyoglobin. Assuming the linear relationship between the adjusted K/S value at 572 nm and the percentage metmyoglobin, it was possible to calculate metmyoglobin as a percentage of the total surface pigments to within ±5%. The ratio of K/S at 572 nm to K/S at 525 nm yielded values of 1.450 ± 0.061 and 0.615 ± 0.050 respectively for 100% and 0% metmyoglobin, enabling the metmyoglobin at the surface to be calculated to within 6 or 7%.

As errors, determined by standard deviations, were greater using the ratio method the adjustment technique was used in the present study. The values obtained were independent of the area of illumination, whether the surface presented to the integrating sphere was flat, convex or concave.

Differences in spectra due to variations in total pigment concentrations are not eliminated by this technique, but calculations showed that differences by a factor of 2 in total concentration should lead to errors within the range of those found experimentally. Pigment concentrations were always within the range 4.6–6.9 mg per g of wet tissue for the different muscles studied.

Another error can arise from the fact that intramuscular fat has a characteristic spectrum. Thus it is unlikely that muscles with different fat contents can be compared by this technique, as the "pigment-free" meats will not yield the approximately parallel reflectance curves (in absorbancy units) that are necessary for this method to be valid. All the spectra determined, on meat of known pigment state, were found to be superimposable, to at least ±4% of R_A in the range 470–650 nm, and so it would appear that variations in the fat contents of the lean muscles studied were not of major importance.

In meat, oxymyoglobin and metmyoglobin have an isosbestic point at 474 nm (Stewart et al., 1965b) and all the above arguments were found to be valid, enabling the percent reduced myoglobin at the surface to be determined. The adjusted K/S values at 474 nm were 2.00 ± 0.09 (range 1.90–2.12) and 3.88 ± 0.10 (range 3.70–4.05) for 100% and 0% of the reduced pigment respectively. This enables percent of reduced myo-

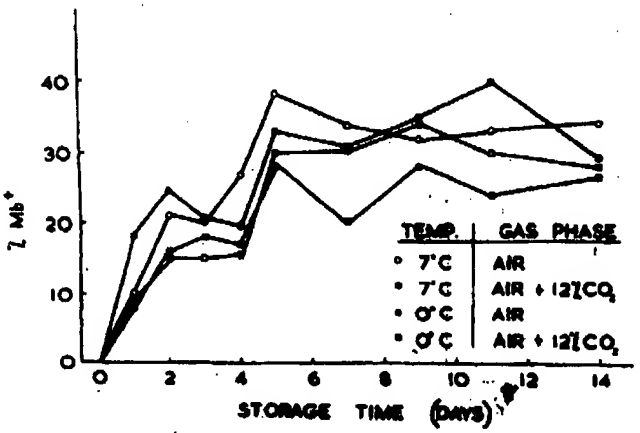

Fig. 1—The effect of 12.0 ± 0.5% CO_2 on the formation of metmyoglobin (Mb^+) at 0°C and 7°C, on a sterile muscle (pH 5.58) at a relative humidity of 99.3%.

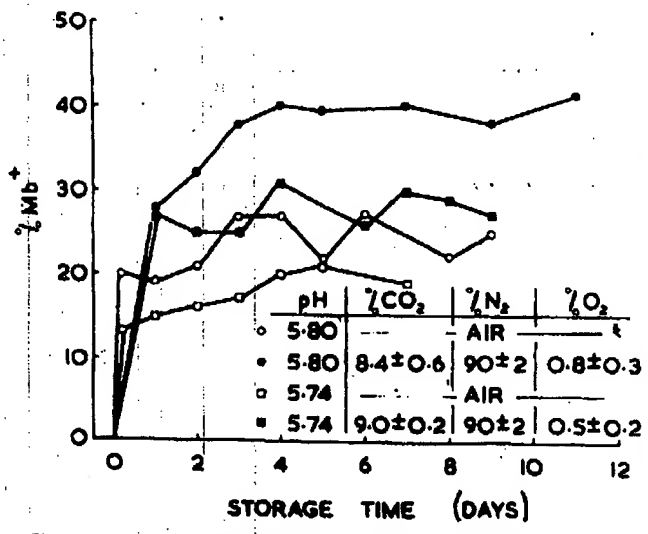

Fig. 2—The formation of Mb^+ at 7°C, on sterile muscles in various atmospheres at a relative humidity of 99.3%. Sampling was discontinued when unavoidable contamination occurred.

globin to be determined to an accuracy of about 5%.

Effect of storage on formation of metmyoglobin

Figure 1 shows the change in surface metmyoglobin concentration as a function of time for samples from the same muscle (pH 5.58) stored in air or 12% CO_2/air at 0 or 7°C and a R.H. of 99.3%. Figure 2 is a similar plot for samples of two muscles (pH 5.80 and 5.74) stored in the atmospheres indicated, at 7°C. At 0°C metmyoglobin formation in these samples was also greater at the lower oxygen partial pressures.

From Figure 1 it is seen that the presence of 12% carbon dioxide had negligible effect upon the formation of metmyoglobin. This was in accord with the observation of Brooks (1933). The increased oxidation found at the lower oxygen partial pressures (Fig. 2) was in general agreement with data obtained by Brooks (1935) and George et al. (1952b) with aqueous hemoglobin and myoglobin solutions.

Figures 1 and 2 both indicate that concentration of metmyoglobin was virtually constant after storage for 5 days.

When the freshly cut meat slices were sealed in the containers, concentration of carbon dioxide rose to between 10% and 15% while concentration of oxygen fell. Equilibrium was established within 48 hr. To ensure equilibrium, samples were stored for 12 ± 2 days, actual storage time governed by practical expediency. In a few instances pressure in the container was reduced by removing a measured volume of gas. In one experiment, muscle was sliced and packed in a nitrogen atmosphere so that the final atmosphere consisted solely of nitrogen and carbon dioxide.

The relationships between metmyoglobin concentration and partial pressure of oxygen, at 7 and 0°C, are summarized in Figures 3 and 4. All values are the means obtained for duplicate samples from the same slice.

Table 1—"Equilibrium" concentration of metmyoglobin for different muscles after storage in air for 12 ± 2 days.

Surface pH of the muscle	"Equilibrium" percent Mb[+] at the surface	
	7°C	0°C
1. 5.58	35 ± 4	31 ± 5
2. 5.60	22 ± 4	18 ± 4
3. 5.60	[2]	33.5 ± 5
4. 5.70	38 ± 5	31.5 ± 5
5. 5.74	20 ± 4	22 ± 4
6. 5.80	26 ± 4	31.5 ± 5
5.70	—	38.5 ± 4[3]
5.72	—	28 ± 3[3]
Av.	28.3 ± 8	Av. 26.3 ± 6

[1] Mb[+] = metmyoglobin.
[2] Not measured—sample contaminated.
[3] Values not included in the average as these muscles were not used in the experiments reported in Figures 3 and 4.

The lower equilibrium concentrations of surface metmyoglobin on samples stored in air, were independent of pH (Table 1).

Brooks (1931) found that rate of oxidation of different samples of ox-blood, at 25°C and constant pH and ionic strength, varied greatly. The present results on meat (Table 1) show similar differences in that the equilibrium concentrations of metmyoglobin vary for different samples, the variation not appearing to be a function of pH. These differences may be explained by differences in rate of enzymatic reduction of metmyoglobin. Stewart et al. (1965b) have shown that the metmyoglobin reducing activity of different samples of ground beef can vary considerably under identical storage conditions.

The data reported by Stewart et al. (1965b) were obtained on metmyoglobin formed by ferricyanide oxidation and may not represent the true reduction of naturally formed metmyoglobin as ferrocyanide forms a complex with ferric heme pigments, this complex catalyzing the enzymatic reduction (Hegesh et al., 1967).

Recently several studies on the enzymatic reduction of ferric heme pigments have been reported and present evidence indicates that NADH plays a vital role (Hegesh et al., 1967), (Watts et al., 1966). Therefore the differences may represent different "NADH ferrihemoglobin and ferrimyoglobin reductase" activities in the muscle. No corrections have been applied to the experimentally determined metmyoglobin concentrations in Figures 3 and 4 to allow for the variations found in the air controls. If, however, corrections were made the fundamental character of plots was unchanged.

Figures 3 and 4 indicate that formation of metmyoglobin was maximal at a partial pressure of oxygen of 7.5 ± 3 mm Hg at 7°C and 6 ± 3 mm Hg at 0°C for the semitendinosus muscles studied. George et al. (1952b) found, for pure myoglobin solutions, that rate of autoxidation was maximal at about 1 mm Hg of oxygen at 30°C and pH 5.69. Brooks (1931) found rate to be maximal at 20 mm Hg of oxygen for ox-

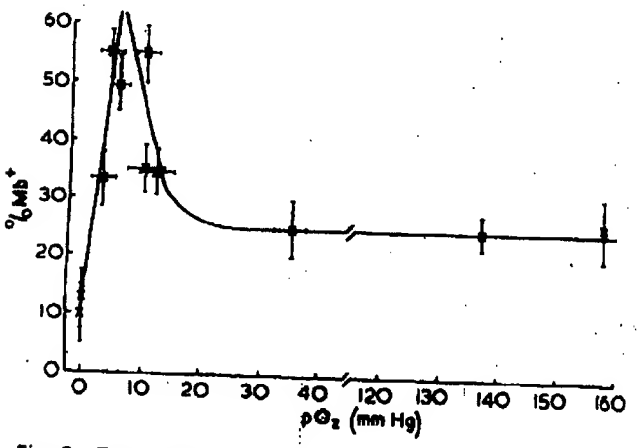

Fig. 3—The relationship between the partial pressure of oxygen and Mb+ formation at the surface of sterile muscles after storage for 12 ± 2 days at 0°C.

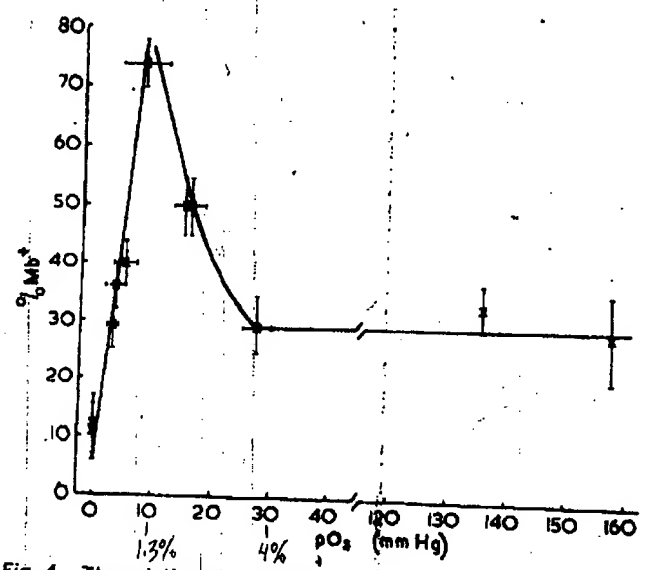

Fig. 4—The relationship between the partial pressure of oxygen and Mb+ formation at the surface of sterile muscles after storage for 12 ± 2 days at 7°C.

Table 2—Effect of CO_2/air concentrations on the formation of metmyoglobin, during 14 days storage, at 0°C.

Pack no.	pCO_2 init. mm Hg	pO_2 init. mm Hg	pCO_2 final mm Hg	pO_2 final mm Hg	ΔpH[1]	Conc. Mb[+] %
1	79	106	63	86	0.03	26.0
2[2]	167	82	46	109	0	29.5
3	190	84	127	84	0.05	32.0
4	273	68	167	74	0.06	32.0
5	312	61	209	68	0.06	31.5
6	403	53	244	64	0.07	31.0
7	471	38	283	59	0.08	36.0
8	578	30	307	50	0.09	40.0
9	669	15	384	41	0.07	63.5 (2.5 hr) 52.0 (24 hr)
10	730	0	535	22	0.12	70.0 (2.5 hr) 51.0 (24 hr)

[1] ΔpH was the increase in surface pH of the samples upon removal from storage; the pH returned to its original value within 2 hr.
[2] Pack 2 leaked slowly with time.

Table 3—Effect of CO_2 pressure on the formation of metmyoglobin at 0°C.

pCO_2 mm Hg	pO_2 mm Hg	ΔpH[1]	"Eq. Conc." of Mb[+]
0	152	—	38.5
76	135	0.06	42.0
190	104	0.05	39.0
380	76	0.06	43.0
510	56	0.10	43.0
650	110	0.27	39.5

[1] ΔpH is as per Table 2.

blood (hemoglobin) at 25°C and pH 5.69.

If the ratio of myoglobin to hemoglobin varied to a marked degree between the muscles studied, a comparison of results would not be valid when expressed as a function of the oxygen partial pressure. It was considered unlikely that such variations occurred. At low oxygen partial pressures, rates of both the autoxidation and enzymatic reductions are increased (Watts et al., 1966). The present results show that increase in rate of oxidation, at 0 and 7°C, is greater than any increase in the enzymatic reduction at the low oxygen pressures studied (Figs. 3 and 4).

This maximal formation of metmyoglobin occurs in all samples, independent of external oxygen pressure, provided this is above the critical value. However, with higher oxygen partial pressures the formation will occur below the surface, the depth at 0°C being about 5 mm in air and varying as the square root of the external oxygen pressure (Brooks, 1929). At the lower oxygen partial pressures the metmyoglobin layer will thus be nearer the surface until, at the critical partial pressure, it is at the surface.

When transferred from the oxygen depleted atmospheres to air, samples with high metmyoglobin contents tended to be reduced with time. This reduction was always very slow, the maximum reduction observed within 24 hr being from 72 to 63% at 7°C with no measurable reduction within this period at 0°C (less than 5% at 55% concentration).

Effect of increased carbon dioxide concentrations

Sterile samples were packed in sealed containers, in atmospheres of various carbon dioxide partial pressures at 0°C. Before packing the samples were left in air for 3–4 hr at 0°C to allow most of the physically bound carbon dioxide to be released. The volume of meat was 25 cc and the total volume 85 cc. In all cases the carbon dioxide concentrations fell due to absorption while the oxygen and nitrogen levels rose. Equilibrium was reached at 24–48 hr. The results are summarized in Table 2.

The values given for the percent of metmyoglobin quoted for the two higher concentrations of carbon dioxide are the values obtained after exposure to air for 2 hr, as reduction occurred over 24 hr (Table 2). All other values are averages of 4 readings, 2 on each of 2 samples, at 2.5 and 24 hr. These results indicate that at the higher carbon dioxide and lower oxygen partial pressures the formation of metmyoglobin increased, presumably due to the decreased oxygen pressures.

The reduction of metmyoglobin found in packs 9 and 10 on exposure to air was greater than any reduction that occurred after storage in 10% carbon dioxide/nitrogen mixtures. Although variations between muscles are to be expected the decrease in pH during storage in high carbon dioxide concentrations (Table 2) may also help to explain this, as autoxidation is accelerated at lower pH values (Brooks, 1931) while enzymatic reduction is retarded (Stewart et al., 1965b).

The initial nonequilibrium of the gas phase in these packs made interpretation of results difficult due to the interrelation between decreased pH and oxygen pressure. In further experiments at 0°C various equilibrium gas phases of carbon dioxide/oxygen were used. The oxygen pressure was always above the level necessary to cause increased metmyoglobin formation. Increasing the carbon dioxide pressure, at 0°C, had no effect on formation of metmyoglobin during 15 days storage even though the pH of the meat was markedly decreased (Table 3).

No measurable change occurred in the surface concentrations of metmyoglobin on re-exposure to air, at the relatively low concentrations of metmyoglobin used in this experiment.

In the first experiment, the meat in higher carbon dioxide atmospheres developed a greyish tinge, which masked the natural "redness" of the meat. This may have been due to decreased pH of the meat causing some of the sarcoplasmic proteins to undergo post-rigor isoelectric precipitation. In the second experiment, even though the muscle was of similar pH (5.72 and 5.70), the meat appeared "normal" at all the carbon dioxide pressures studied. In subsequent experiments this "greying" phenomenon has been observed in samples stored for 14 and 28 days in 60% carbon dioxide atmospheres, but no explanation can be offered for its occurrence in only certain muscles.

From the results described in the present paper it would appear that the storage of sterile meat in carbon dioxide enriched atmospheres leads to no increased metmyoglobin formation, provided the oxygen partial pressure is maintained above a limiting value of about 5%.

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Comparison of Carbonyl Compounds in Moldy and Non-moldy Cocoa Beans

SUMMARY—Carbonyl compounds in moldy and non-moldy cocoa beans were converted to dinitrophenylhydrazones and separated into monocarbonyl classes. Growth of mold was always accompanied by relatively large increases in carbonyl concentrations. Increases in total monocarbonyl values ranged from 20 to 500% and averaged almost 300% for the eight pairs of samples analyzed. Compared to non-moldy beans, moldy cocoa beans contained greater concentrations of methyl ketones, 2-enals and 2,4-dienals, but saturated aldehyde concentrations were quite often lower. TLC revealed the presence of C_8, C_9, C_{10} and C_{11} and several unidentified methyl ketones. Most of the ketones detected in moldy beans were also found in non-moldy beans but in lower concentrations. Qualitatively, the unsaturated aldehyde fractions varied considerably among samples. 2-Pentenal and 2,4-pentadienal appeared as prominent TLC spots and other 2-enals and 2,4-dienals were frequently observed in moldy beans. The only unsaturated aldehydes detected in non-moldy beans by TLC were 2-pentenal and 2,4-octadienal.

INTRODUCTION

CONDITIONS for mold attack in the tropics are favorable in damaged or improperly dried cocoa beans. It would be expected that beans are normally highly infested with mold spores and with an increase in moisture these spores may germinate and cause undesirable changes to occur.

Boyd, et al. (1965) in a study of the monocarbonyls of chocolate suggested that mold activity might have contributed significantly to the observed variation in methyl ketone content of cocoa beans. Unfortunately, these investigators did not have an opportunity to collect supporting data by analyzing moldy cocoa beans. It was envisioned that if Boyd's suggestion could be verified, a way might be opened for the development of an objective chemical procedure to replace the current subjective "cut test" for determining mold infestation. At present the cut test involves cutting the cocoa bean longitudinally and visually observing for mold.

The research reported in this paper concerns the differences in total carbonyls, total monocarbonyls and relatively concentrations of the different monocarbonyl classes in moldy and non-moldy cocoa beans. The data collected were based on the methods of Schwartz, et al. (1963) as modified for chocolate products by Boyd, et al. (1965). The techniques involved the conversion of carbonyls to 2,4-dinitrophenylhydrazones (DNP-hydrazones) followed by separation into the following classes: methyl ketones, saturated aldehydes, 2-enals and 2,4-dienals. Thin layer chromatography procedures of Schwartz, et al. (1968) were used to identify and assess the complexity of each class of monocarbonyls.

EXPERIMENTAL

Samples

Several samples of moldy cocoa beans, supplied by chocolate manufacturers, yielded unusually high carbonyl values. Since many variables affect carbonyl concentrations (Boyd, 1965), it was decided that the best experimental approach was to develop mold on beans under controlled laboratory conditions. This made possible comparison of carbonyl values before and after growth of mold, thereby minimizing the effect of many interfering variables.

Aspergillus and *Penicillium* were the two main types of mold isolated from cocoa beans and were used to inoculate non-moldy beans for control-molded samples. The *Aspergillus* and *Penicillium* spores were removed from moldy cocoa beans with sterile water and then inoculated onto cocoa beans using a sterile, platinum loop. The inoculated cocoa beans were placed in a 1 qt polyethylene container with a small mat of filter paper (1 in. × 1 in. × 1/4 in.) saturated with sterile water to induce mold growth. The beans were stored at approximately 24°C to facilitate mold growth and simulate tropical conditions. Cocoa beans from several of the major producing countries were included in the study.

Moldy beans were also obtained directly from the tropics through the cooperation of the Turrialba Experiment Station, Costa Rica. A batch of fermented Matina beans had been split and one portion dried under normal conditions while the other half was purposely kept from drying for several days to allow mold to grow. These samples made it possible to determine the changes which take place as a result of mold growth during the fermenting and drying stages.

Solvents

All solvents were freshly distilled and rendered carbonyl free (Schwartz, et al.,